



Electrochemical Determination of Amitriptyline using a Nanocomposite Carbon Paste Electrode in Human Body Fluids

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Abstract

A carbon nanocomposite electrode has been designed by incorporation of montmorillonite nanoclay into the carbon ionic liquid electrode for the electrochemical determination of amitriptyline. The surface of the proposed electrode was characterized by scanning electron microscopy. The presence of ionic liquid as a binder is believed to be responsible for the electrocatalytic activity of the proposed electrode for oxidation of amitriptyline. Low detection limit of 24 nM for amitriptyline was achieved. High stability and reproducibility as well as the ease of preparation, low cost and surface renewal made this electrode ideal for the determination of amitriptyline in human body fluids.

Keywords: Amitriptyline, Carbon ionic liquid electrode, Electrochemistry, Nanoclay

1. Introduction

Amitriptyline (AMT) belongs to the tricyclic group of antidepressants and is prescribed in adults to treat neuropathic pain and migraine. AMT can be prescribed to children to treat nocturnal enuresis, i.e., involuntary passing of urine (bed-wetting). If taken in excess, AMT may cause coma, seizures and abnormal heart rhythms [1]. Therefore, the determination of this compound becomes an important analytical task. Electrochemical methods may be used for this purpose due to their excellent sensitivity, short analysis time, simplicity, and the low costs involved

in the implementation of these powerful tools for measuring different analytes. Only a few reports are present in the literature on the electrochemical measurements of AMT, in which they were investigated by highly boron-doped diamond electrodes (BDE) [2], and functionalized carbon paste electrodes [3,4].

Electrode modification with clays brings the benefits of ionic exchange capacity, good catalytic support, large surface area, mechanical stability, low cost, environmentally friendly analytical protocols and devices, negligible waste, and nontoxic materials for wide applications in electrochemistry [5-7]. Clays are also mixed with carbon paste to enhance the adsorptive and ion-exchange properties of the electrode [8]. Montmorillonite nanoclay with

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its inherent layered inorganic nanostructure is a member in the smectite group of clays [9, 10] and has a high affinity for several substances (e.g., heavy metals and organic molecules) [10, 11]. In recent years, carbon ionic liquid electrodes (CILEs), with the advantages low cost, ease of preparation, high conductivity, wide electrochemical windows, antifouling effect and renewable surface, have been widely used in the field of electrochemical sensing [12]. CILE can be prepared by substituting the nonconductive organic binder with high conductive ionic liquids (ILs), which exhibit superior electrochemical performances over the traditional working electrodes [13].

The interaction of ILs and clays were studied and they were applied as a suitable matrix for immobilization of different biomolecules on the surface of electrodes [11, 14, 15]. In this work the nanocomposite electrode was applied for electrochemical detection of AMT. The AMT was accumulated on the surface of electrode through the cooperative interaction of IL and clay with the AMT for subsequent enhanced determination. The results illustrate that the electrode exhibits an excellent route to determination of AMT.

2. Experimental

2.1 Chemicals and Apparatus

Amitriptyline hydrochloride, (AMT- HCl) (1-propanamine, 3-(10, 11-dihydro-5H-dibenzo [a,d]cyclohepten-5-ylidene) N,N-Dimethyl -hydrochloride) pyridine, diethyl ether, paraffin oil (Merck), montmorillonite nanoclay, graphite powder (particle size <100 nm) (Aldrich), ammonium hexafluorophosphate and iodoctane (Fluka) were used as received. All other chemicals were of analytical grade. The IL, octylpyridinium iodide, was synthesized as described

elsewhere [16]. Deionized distilled water was used to prepare all solutions. Britton-Robinson buffer solution was used to study the effect of pH on the analytical signal. Phosphate buffer solution (PBS 0.07 M, pH 7.2) was used as supporting electrolyte. Daily-based fresh frozen blood serum samples were prepared from the venous blood of random healthy male and female blood donors, obtained from Central Blood Transfusion Organization (Shiraz, Iran). Urine sample obtained from healthy individuals were stored frozen until assay. Electrochemical measurements were done with a galvano potentiostat Behpajoo Co. model BHP2063+. The electrochemical cell was assembled with a conventional three-electrode system; with a saturated calomel electrode as a reference electrode (SCE) and a platinum disk as a counter electrode. The cell was a one-compartment cell with an internal volume of 10 mL. All experiments were typically conducted at room temperature. Scanning electron microscopy (SEM) images were observed using SEM (Philips XL 30 and S-4160) with gold coating equipped with energy dispersive X-ray spectroscopy (EDX).

2.2. Electrode Preparation

CILE was prepared by hand-mixing, in a mortar, the graphite powder and IL ([OPy]⁺[PF₆]⁻) in a mortar with a ratio of 70/30 (w/w). A portion of the resulting paste was packed firmly into the cavity (2.0 mm i.d.) of a Teflon holder. Note that in order to have better homogeneity in the composite and to lower background current, the electrode should be heated (e.g. by using a hair drier) to a temperature above the melting point of IL (m.p. ~65 °C) prior to use [8]. The electric contact was established with a copper wire contact to the carbon composite. Nanoclay modified carbon ionic liquid electrode (NC-CILE) was

prepared in the same way as CILE with the weighed amounts of graphite powder, IL, and nanoclay (60 :30 :10,wt%), respectively. The unmodified carbon-paste electrode (CPE) was prepared by mixing graphite powder and appropriate amount of paraffin oil with a ratio of 70/30 (w/w) graphite/paraffin oil. The paste was packed into the cavity of a Teflon tube (2-mm diameter). Nanoclay modified carbon paste electrode (NC-CPE) was prepared in the same way as CPE with the weighed amount of graphite powder, nanoclay and paraffin oil (60:10 : 30, wt %).

3. Results and discussion

3.1. Characterization of NC-CILE

Figure 1 shows SEM micrographs of nanoclays (as received) (A) and Nanoclay modified carbon ionic liquid electrode (NC-CILE) (B). As it can be seen, nanoclays are in the form of large and small aggregates. However, after the incorporation of nanoclays into the CILE, nanoclay particles are dispersed in the form of homogeneous nanostructures with a dimension about 30 nm. Certainly, the narrowly dispersed nanoparticles enhance the contact surface area of the clays with the solution.

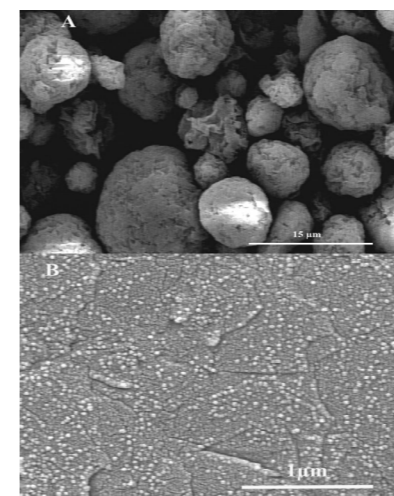


Fig.1. SEM micrographs of nanoclays as received (A), Nanoclay modified carbon ionic liquid electrode (NC-CILE) (B).

3.2. Electrochemical characterization of AMT on the surface of CILE , NC-CILE, CPE and NC- CPE

The cyclic voltammograms of 0.1 mM AMT in 0.07 M PBS (pH 7.2) obtained at a potential sweep rate of 50 mVs⁻¹ at CILE, NC-CILE (10%, wt %), CPE and NC-CPE (10%-wt%) are given in Figure 2. None of these electrodes show any peak in the absence of AMT (curves a, b, c, d). The decrease in overpotential due to electrocatalytic activity of CILE is more pronounced for the oxidation of AMT. It can be seen from Figure 2 that the oxidation peak potential of AMT on both CILE (curve g) and NC-CILE (curve h) is shifted to a more negative potential (0.65 V vs. SCE) compared to CPE (curve e), NC-CPE (curve f) and other conventional solid electrodes (0.9 V at glassy carbon electrode (GCE) and 1.3 V at BDE) [2]. The superiority of IL modified electrodes can be attributed to their inherent electrocatalytic activity which is the consequence of substituting the traditional liquid paraffin with ILs as it was reported previously [17, 18]. It is interesting to note that an increase in faradic currents (increase in the sensitivity) for AMT at CILE and NC-CILE in comparison with classic CPE and NC-CPE

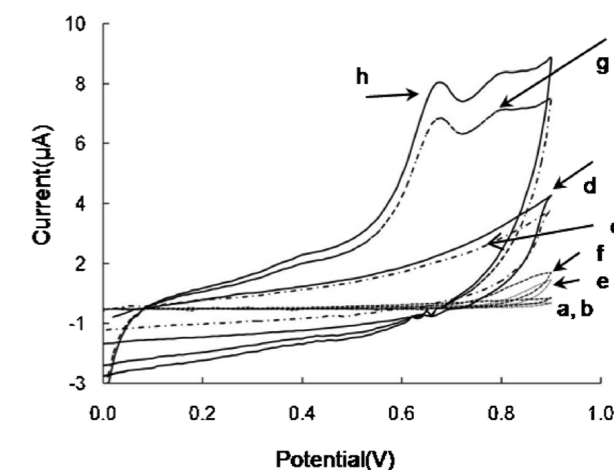


Fig.2. Cyclic voltammograms of (a) CPE, (b) NC-CPE, (c) CILE, (d) NC-CILE in the absence and (e) CPE, (f) NC- CPE, (g) CILE, (h) NC-CILE in the presence of 0.1 mM AMT in PBS 0.07 M (pH 7.2) at a scan rate of 50 mVs⁻¹.

is caused by ionic conductivity of the binder which leads to a larger electroactive area. This is because before electron transfer occurs, some fraction of polar reactant is transferred across IL/aqueous electrolyte and electrode reaction takes place at carbon/IL interface within CILE body. This is not possible at classic CPE with nonpolar binder, where electrode reaction occurs at carbon particles surface next to aqueous electrolyte [19]. On the other hand, montmorillonite possesses a physical structure consisting of sheets of aluminosilicates [20] which is neutralized by the intercalation of compensating, exchangeable Na^+ . The Na^+ ion can be exchanged with a wide variety of hydrated inorganic cations or organic cations [21]. Exchange of Na^+ with the cations of the ILs have been reported in the literature [20]. Since AMT is protonated and has positive charge in the working solution (phosphate buffer, pH 7.2), The interaction with montmorillonite nanoclay is similar to the other organic cationic species in this matrix [22, 23]. Moreover the peak currents for AMT oxidation increased with time and reached a relatively constant value after several minutes (~240 s). Therefore, NC-CILE was selected for further studies.

3.3. Effect of pH

Cyclic voltammograms of 0.1 mM AMT obtained in the pH range of 4–7.2 employing the Britton_Robinson (B. R.) buffer and the results were presented in Figure 3. The oxidation peak current of AMT was not observed below pH 4. As seen, with solution pH raising the peak current increased and reached to the maximum value at pH 7.2. Since the pH 7.2 PBS gave the same response in terms of the peak current and the peak shape for AMT, PBS with pH 7.2 was used as the supporting electrolyte in all voltammetric

determinations. From the plot of oxidation peak potential (E_p) vs. pH (Fig.3, inset) for AMT (E_p (V) = $1.081 - 0.059 \text{ pH}$) the slope of -0.059 V/pH was observed. It was reported that the electrooxidation of AMI takes place at the nitrogen atom in the alkylamine with loss of one electron, resulting in the formation of a cation radical, followed by deprotonation [24]. The pH dependence of the voltammetric peak current with the slope of -0.059 V/pH showed participation of equal numbers of protons and electrons in the oxidation reaction and confirmed the reported mechanism [24].

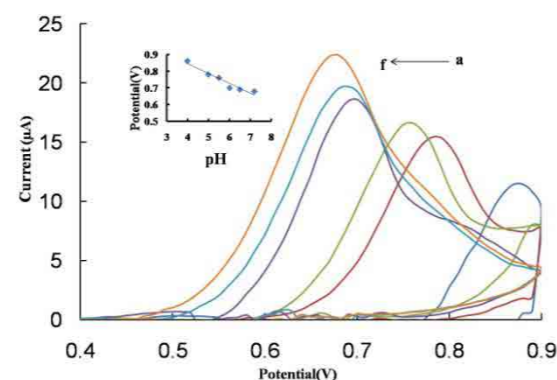


Fig. 3. Background subtracted cyclic voltammograms of 0.1 mM AMT at the NC-CILE; pH from a to f: 4.0, 5.0, 5.5, 6.0, 6.5, 7.2 at 50 mVs^{-1} , accumulation time 240 s and accumulation potential 0 V. Inset: plot of oxidation peak potential vs. pH.

3.4. Effect of modifier

The influence of the amounts of nanoclay on the voltammetric response of the NC-CILE was evaluated. Four electrodes containing different amounts of nanoclay (2.5, 5, 10 and 15, % weight percent ratio) were prepared and examined for their voltammetric signals under identical conditions. Maximum peak current for AMT was obtained for 10% modifier in the CILE. The results indicated that by increasing the amount of modifier up to 10% the anodic peak current increased, whereas higher content of the modifier caused a decrease in the peak current (Fig. 4). This is because the sites for adsorption increased with the increase of

nanoclay percentage in the modified electrode, while the excess of nanoclay increases the resistance of the electrode which results in enhanced resistance to the electron transfer. Consequently a NC-CILE (10%, w/w) was used in further studies.

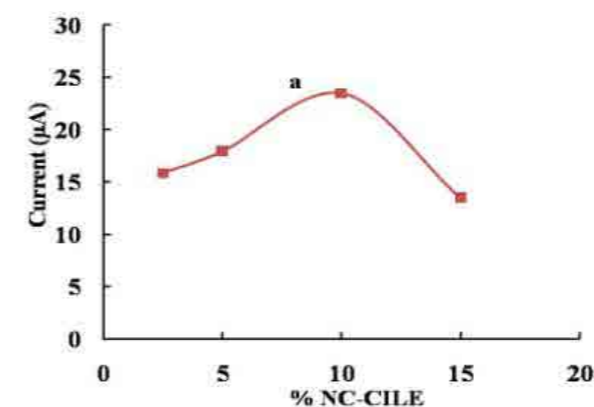


Fig. 4. The influence of % NC-CILE on the anodic peak current of 0.1 mM AMT in PBS 0.07 M (pH 7.2) at a scan rate of 50 mVs^{-1} .

3.5. Effect of accumulation potential and accumulation time

The accumulation potential as well as accumulation time is an effective factor which affects the response sensitivity. The effect of accumulation potential on the peak current of AMT was examined over the range of -300 to 500 mV keeping the accumulation time 240 s. The oxidation peak current increased up to 0 V. Therefore, an optimal accumulation potential of 0 V was used for further studies. The accumulation time was changed from 0 to 300 s employing optimized accumulation potential value. It was observed that the peak current increased with accumulation time, the more AMT was adsorbed and thus the peak currents became larger, and reached to a constant value after a certain accumulation period. Therefore, An accumulation time of 240 s was chosen for further experiments.

3.6. Effect of scan rate

Cyclic voltammetry for AMT was carried

out at different scan rates (Fig. 5). Results indicated that there is a linear relationship between the peak current (i_p) of AMT and the scan rate (v) in the range of 10–100 mV/s (Fig. 5, inset). With scan rate increasing, the anodic peak of the NC-CILE grew and the oxidation peak potential shifted to more positive potentials confirming the kinetic limitation in the electrochemical reaction [25]. A linear correlation was also obtained between the log (peak current) vs. log (scan rate) with a slope of 0.806 (correlation coefficient, 0.993) indicating that the AMT electrooxidation reaction is adsorption controlled.

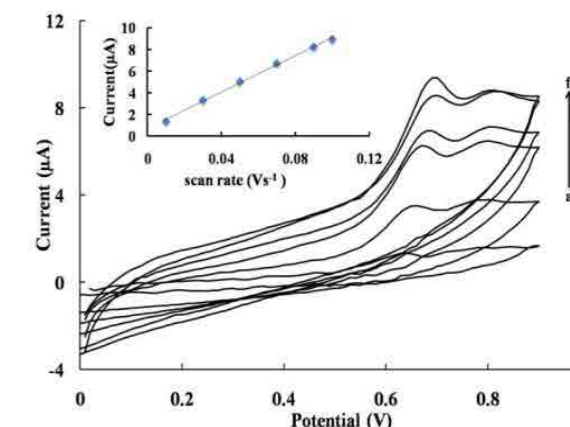


Fig. 5. Cyclic voltammograms of NC-CILE in 0.07M PBS (pH 7.20) containing 0.1 mM AMT at various scan rates: a-f ($10-100 \text{ mVs}^{-1}$). Inset: plot of peak current vs. scan rate.

3.7. Linear range and limit of detection

Under optimum conditions, the electrochemical reactions of different concentration of AMT after 240 s accumulation time have been studied and the cyclic voltammograms are shown in Figure 6. The calibration graph of current (Fig. 6, inset) was linearly related to AMT concentration over the ranges of $0.1-8 \mu\text{M}$, with the regression equation of $I_p (\mu\text{A}) = 0.748 + 0.853C (\mu\text{M})$ and $8-40 \mu\text{M}$ with the regression equation of $I_p (\mu\text{A}) = 0.321 + 4.299C (\mu\text{M})$ with correlation coefficients of 0.995 and 0.992, respectively,

The limit of detection (signal-to-noise ratio of 3) was 24nM. The electrochemical responses of the NC-CILE in terms of linear range and detection limits, were compared to the other modified electrodes, which were reported in the literature (Table 1) [2, 4]. As stated before, the electrochemical determination of AMT was rarely reported. However, the NC-CILE can be applied conveniently for this purpose with lower detection limit in comparison with BDE [2] and polymer modified CPE [4].

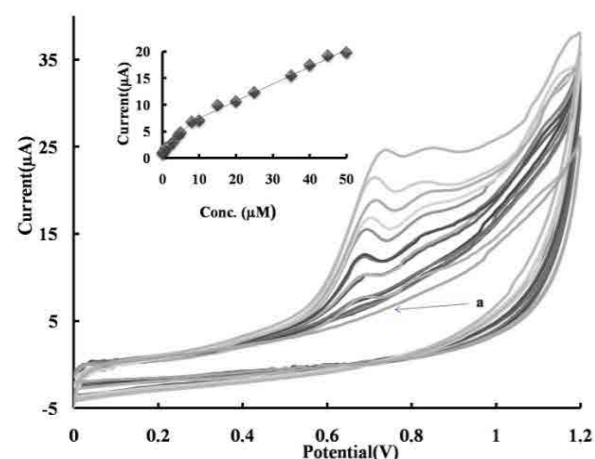


Fig. 6. Cyclic voltammograms of NC-CILE in 0.07 M PBS, pH 7.2 in the presence and (a) in the absence of different concentrations of AMT measured, accumulation time 240s and accumulation potential 0 V. Inset shows the calibration curve of peak current vs. AMT concentration.

3.8. Stability, repeatability and reproducibility of the electrode

The stability of the proposed nanocomposite electrode was checked by recording the response of the electrode in 2 µM of AMT after every few days. The NC-

CILE shows high stability for AMT detection and retains 88.05% of its original response to AMT after 30 days of storage. The repeatability was estimated through the relative standard deviation of 7 replicate measurements of 2 µM of AMT in 0.07 M PBS (pH 7.2). The relative standard deviations (RSDs) of 2.8% for AMT revealed good repeatability of the fabricated electrode for determination of AMT. The responses of four similar electrodes were measured separately toward 2 µM AMT, and RSDs of 3.17%, was obtained confirming high reproducibility of the proposed method.

The response of the proposed composite electrode was evaluated toward some common species found in biological fluids such as glucose, ascorbic acid, uric acid. The tolerance limit was defined as the maximum concentration ratio of interfere/ AMT causing an error less than $\pm 5.0\%$ for the determination of AMT. In the presence of 10 µM AMT the results showed that 100-fold excess of glucose; 50-fold excess of ascorbic acid and 10-fold excess of uric acid; did not interfere with the analysis of AMT. The results demonstrated good selectivity for the proposed electrode.

3.9. Real sample analysis

In order to demonstrate the ability of the NC-CILE to determine AMT concentration, human serum and urine samples were used. The Blood serum sample was deproteinized by adding 2 ml of 10% (w/w) trichloroacetic acid solution to 10 ml sample and the solution was centrifuged then the sample was diluted

10 times with 0.07 M PBS (pH 7.2) then appropriate amounts of this diluted sample was transferred to the electrochemical cell for the determination of AMT. The results are presented in Table 2.

Table 2. Determination of AMT in body fluids using the proposed method (n=3).

Sample	Added (µM)	Found (µM)	Recovery (%)
Blood serum	0	N.D.*	-
	0.5	0.53(±0.02)	106.0
	1.0	0.92(±0.03)	92.0
	1.5	1.53(±0.03)	102.0
Urine	0	N.D.*	-
	0.5	0.52(±0.03)	104.0
	1.0	0.95(±0.03)	95.0
	1.5	1.52(±0.05)	101.3

* Not detected

Urine sample was also diluted with 0.07 M PBS (pH 7.2) and then appropriate amount of this diluted sample was transferred to the electrochemical cell for the determination of AMT (Table 2). Recovery results were not affected significantly and consequently the described method is accurate for the assay of AMT in human serum and urine samples.

4. Conclusion

In this work, CILE modified with montmorillonite nanoclay (NC-CILE) was demonstrated as effective electrode for the determination of AMT. An effective accumulation of the drug molecules has been found on the electrode due to synergistic effect of nanoclays and IL. High stability and reproducibility as well as the ease of preparation, low cost and surface renewal made this electrode ideal for the determination of AMT in human body fluids.

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Table 1. Electrochemical detection of AMT reported at various electrodes. PNI-CPE: poly(N-vinylimidazole) modified CPE.

Modified electrodes	Linear range (µM)	Detection limit(nM)	Reference
BDE	0.05 - 100	163	[2]
PNI-CPE	10-100	-	[4]
NC - CILE	0.1-8 8-50	24	This work